

CLAIMS:

1. A non-naturally occurring protein which inhibits human neutrophil elastase and which is a protein comprising at least the core sequence of a non-naturally occurring Kunitz domain, said Kunitz domain being more similar in sequence to the core sequence 26-76 of ITI-D1 than to the core sequence 5-55 of BPTI, when its cysteines are aligned with those of BPTI and ITI-D1, but said domain differing from ITI-D1 in that at least one of the following conditions applies:

- (a) the residue corresponding to BPTI residue 15 and ITI-D1 residue M36 is Val or Ile,
- (b) the residue corresponding to BPTI residue 16 and ITI-D1 residue G37 is Ala,
- (c) the residue corresponding to BPTI residue 18 and ITI-D1 residue T39 is Phe,
- (d) the residue corresponding to BPTI residue 19 and ITI-D1 residue S40 is Pro,
- (e) the residue corresponding to BPTI residue 1 and ITI-D1 residue K22, if any, is Arg,
- (f) the residue corresponding to BPTI residue 2 and ITI-D1 residue E23, if any, is Pro, or
- (g) the residue corresponding to BPTI residue 4 and ITI-D1 residue S25, if any, is Phe.

2. The protein of claim 1 which differs from human ITI-D1 at least one of the positions corresponding to BPTI positions 15-20.

3. The protein of claim 1 where, in said Kunitz domain, BPTI positions 1-4 are Arg-Pro-Asp-Phe (residues 1-4 of SEQ ID NO:17).

4. The protein of claim 1 where the said Kunitz domain the residue corresponding to BPTI position 31 is Glu.

5. The protein of claim 1 where the said Kunitz domain the residue corresponding to BPTI position 31 is Gln.

6. The protein of claim 1 where the said Kunitz domain the residue corresponding to BPTI position 34 is Val.

7. The protein of claim 1 where in said Kunitz domain the residue corresponding to BPTI position 4 is Phe.

8. The protein of claim 1 where in said Kunitz domain the residue corresponding to BPTI position 2 is Pro.

9. The protein of claim 1 where the said Kunitz domain the residue corresponding to BPTI position 1 is Arg.

10. The protein of claim 1 where the said Kunitz domain the residue corresponding to BPTI position 26 is Ala.

11. The protein of claim 1 where the said Kunitz domain the residue corresponding to BPTI position 18 is Phe.

12. The protein of claim 1 where in said Kunitz domain the residue corresponding to BPTI position 15 is Val or Ile, 16 is Ala or Gly, 17 is Met or Phe and 19 is Pro or Ser.

13. The protein of claim 1 which has an affinity for HNE such that its K_D is less than 10^{-8} M.

14. The protein of claim 1 which has an affinity for HNE such that its K_D is less than 10^{-9} M.

15. The protein of claim 1 which has an affinity for HNE such that its K_D is less than 10^{-11} M.

16. The protein of claim 1 wherein both conditions (a) and (c) apply.

17. The protein of claim 16 wherein condition (d) also applies.

18. The protein of claim 1 wherein conditions (e)-(g) apply.

19. The protein of claim 16 wherein conditions (e)-(g) also apply.

20. The protein of claim 17 wherein conditions (e)-(g) also apply.

21. The protein of claim 1 where said Kunitz domain is a reference domain selected from the group consisting of BITI-E7-1222, AMINO1 (SEQ ID NO:22), AMINO2 (SEQ ID NO:23), MUTP1 (SEQ ID NO:24), BITI-E7-141 (SEQ ID NO:17), MUTT26A (SEQ ID NO:18), MUTQE (SEQ ID NO:19), and MUT1619 (SEQ ID NO:20) or a Kunitz domain comprising an amino acid sequence which otherwise differs from the core sequence of one or more of said reference domains solely by one or more class A and/or one or more class B substitutions as set forth in Table 65.

22. The protein of claim 1 where said non-naturally

occurring Kunitz domain is a reference domain selected from the group consisting of BITI-E7-1222, AMINO1, AMINO2, MUTP1, BITI-E7-141, MUTT26A, MUTQE, and MUT1619 in Table 220 or a kunitz domain comprising an amino acid sequence which differs from the core sequence of one or more of said reference domains solely by one or more class A substitutions as set forth in Table 65.

23. The protein of claim 1 where the core sequence of said Kunitz domain consists of an amino acid sequence identical to that of the core sequence of a reference domain selected from the group consisting of BITI-E7-1222, AMINO1, AMINO2, MUTP1, BITI-E7-141, MUTT26A, MUTQE, and MUT1619 in Table 220.

24. The protein of claim 1 where said Kunitz domain is selected from the group consisting of BITI-E7-1222, AMINO1, AMINO2, MUTP1, BITI-E7-141, MUTT26A, MUTQE, and MUT1619 in Table 220.

25. The protein of claim 24 where said protein further comprises at least a functional portion of a coat protein of a filamentous phage, sufficient to cause display of said protein on the surface of a filamentous phage particle if said protein is expressed, together with the other proteins of said phage, in a cell capable of assembling said particles.

26. The protein of claim 25 where said coat protein is the one corresponding in said filamentous phage to the gene III protein of M13 phage.

27. The protein of claim 1 which is identical to a protein selected from the group consisting of BITI-E7-1222, AMINO1, AMINO2, MUTP1, BITI-E7-141, MUTT26A, MUTQE, and MUT1619 in Table 220.

28. The protein of claim 1 where said protein is BITI-E7-141.

29. The protein of claim 1 where said protein is MUTT26A (SEQ ID NO:18).

30. The protein of claim 1 where said protein is MUTQE (SEQ ID NO:19).

31. The protein of claim 1 where said protein is

MUT1619 (SEQ ID NO:20).

32. The protein of claim 1 where said Kunitz domain is not identical in amino acid sequence to any of the Kunitz domain amino acid sequences set forth in Table 13.

5 33. A method of inhibiting human neutrophil elastase (HNE) which comprises contacting the HNE with an inhibitor effective amount of a protein of any one of claims 1, 12, and 14-23.

10 34. A method of inhibiting harmful human neutrophil elastase activity in a subject which comprises administering to the subject an inhibitorily effective amount of a protein of any one of claims 1, 12 and 14-23.

15 35. A method of treating emphysema in a subject which comprises administering to the subject a therapeutically effective amount of a protein of claim 1.

20 36. A method of treating cystic fibrosis in a subject which comprises administering to the subject a therapeutically effective amount of a protein of claim 1.